

WHAT IS CLAIMED IS:

1. A method for refolding a small, disulfide-rich peptide comprising:
  - (a) adding a peptide which comprises 5-55 amino acid residues containing two or more cysteines which form disulfide bonds to a refolding mixture which comprises
    - (i) a non-ionic detergent and
    - (ii) a redox reagent
  - (b) incubating the resulting mixture to form disulfide bonds in said peptide, whereby a refolded peptide is produced.
2. The method of claim 1, wherein said refolding mixture further comprises a cosolvent.
3. The method of claim 1, wherein the non-ionic detergent is selected from the group consisting of polyoxyethylenes, polyoxyethylene derivatives, alkyl derivatives of carbohydrates and mixtures thereof.
4. The method of claim 3, wherein said polyoxyethylene derviatives are selected from the group consisting of polyoxylethylene sorbitans, polyoxylethylene ethers and polyoxylethylene esters.
5. The method of claim 3, wherein said carbohydrate is selected from the group consisting of glucose and maltose.
6. The method of claim 2, wherein said cosolvent is selected from the group consisting of (i) methanol, (ii) ethanol, (iii) isopropanol, (iv) acetonitrile, (v) a solvent selected from the group consisting of primary, secondary, tertiary, allylic, benzylic alcohols, ethers, aldehydes, ketones, carboxylic acids, amines, poly- and heterocyclic aromatic compounds, and (vi) mixtures thereof.
7. The method of claim 1, wherein said redox reagent is selected from the group consisting of oxidized glutathione, reduced glutathione, cystine, cysteine, cystamine,  $\beta$ -mercaptoethanol and 2-hydroxyethyl disulfide.

8. The method of claim 1, wherein said redox reagent comprises an oxidizing agent and a reducing agent.
9. The method of claim 7, wherein said redox reagent comprises an oxidizing agent and a reducing agent.
10. The method of claim 1, wherein the refolding is performed at a temperature in the range of  $-10^{\circ}\text{C}$  to  $60^{\circ}\text{C}$ .
11. The method of claim 1, wherein the refolding is performed at a pH in the range of 5 to 12.
12. The method of claim 1, wherein the peptide is immobilized on a solid support.
13. A method for refolding a small, disulfide-rich peptide comprising:  
(a) adding a peptide which comprises 5-55 amino acid residues containing two or more cysteines which form disulfide bonds at a concentration from about  $0.1\text{ }\mu\text{M}$  to about  $100\text{ mM}$  to a refolding mixture which comprises  
(i) a non-ionic detergent in an amount from about  $0.001\%$  to about  $90\%$  and  
(ii) a redox reagent in an amount from about  $0.01\text{ mM}$  to about  $25\text{ mM}$   
(b) incubating the resulting mixture to form disulfide bonds in said peptide, whereby a refolded peptide is produced.
14. The method of claim 13, wherein said refolding mixture further comprises a cosolvent in an amount from about  $0.1\%$  to about  $90\%$ .
15. The method of claim 13, wherein the non-ionic detergent is selected from the group consisting of polyoxyethylenes, polyoxyethylene derivatives, alkyl derivatives of carbohydrates and mixtures thereof.
16. The method of claim 15, wherein said polyoxyethylene derviatives are selected from the group consisting of polyoxylethylene sorbitans, polyoxylethylene ethers and polyoxylethylene esters.

17. The method of claim 15, wherein said carbohydrate is selected from the group consisting of glucose and maltose.
- 5 18. The method of claim 14, wherein said cosolvent is selected from the group consisting of (i) methanol, (ii) ethanol, (iii) isopropanol, (iv) acetonitrile, (v) a solvent selected from the group consisting of primary, secondary, tertiary, allylic, benzylic alcohols, ethers, aldehydes, ketones, carboxylic acids, amines, poly- and heterocyclic aromatic compounds, and (vi) mixtures thereof.
- 10 19. The method of claim 13, wherein said redox reagent is selected from the group consisting of oxidized glutathione, reduced glutathione, cystine, cysteine, cystamine,  $\beta$ -mercaptoethanol and 2-hydroxyethyl disulfide.
- 15 20. The method of claim 13, wherein said redox reagent comprises an oxidizing agent and a reducing agent.
- 20 21. The method of claim 20, wherein said redox reagent comprises an oxidizing agent and a reducing agent.
22. The method of claim 13, wherein the refolding is performed at a temperature in the range of  $-10^{\circ}\text{C}$  to  $60^{\circ}\text{C}$ .
- 25 23. The method of claim 13, wherein the refolding is performed at a pH in the range of 5 to 12.
24. The method of claim 13, wherein the peptide is immobilized on a solid support.
- 30 25. A method for preparing a small, disulfide rich peptide having a disulfide bridging pattern of a native peptide comprising:  
(a) synthesizing a peptide which comprises 5-55 amino acid residues containing two or more cysteines which form disulfide bonds;  
(b) isolating the synthesized peptide;

(c) adding the peptide to a refolding mixture which comprises

(i) a non-ionic detergent and

(ii) a redox reagent

(d) incubating the resulting mixture to form disulfide bonds in said peptide; and

(e) isolating the refolded peptide.

26. The method of claim 25, wherein the peptide is synthesized by chemical synthesis.

27. The method of claim 25, wherein the peptide is synthesized by a recombinant DNA technique.

28. The method of claim 25, wherein said refolding mixture further comprises a cosolvent.

29. The method of claim 25, wherein the non-ionic detergent is selected from the group consisting of polyoxyethylenes, polyoxyethylene derivatives, alkyl derivatives of carbohydrates and mixtures thereof.

30. The method of claim 29, wherein said polyoxyethylene derivatives are selected from the group consisting of polyoxyethylene sorbitans, polyoxyethylene ethers and polyoxyethylene esters.

31. The method of claim 29, wherein said carbohydrate is selected from the group consisting of glucose and maltose.

32. The method of claim 28, wherein said cosolvent is selected from the group consisting of (i) methanol, (ii) ethanol, (iii) isopropanol, (iv) acetonitrile, (v) a solvent selected from the group consisting of primary, secondary, tertiary, allylic, benzylic alcohols, ethers, aldehydes, ketones, carboxylic acids, amines, poly- and heterocyclic aromatic compounds, and (vi) mixtures thereof.

33. The method of claim 25, wherein said redox reagent is selected from the group consisting of oxidized glutathione, reduced glutathione, cystine, cysteine, cystamine,  $\beta$ -mercaptoethanol and 2-hydroxyethyl disulfide.

34. The method of claim 25, wherein said redox reagent comprises an oxidizing agent and a reducing agent.

5 35. The method of claim 33, wherein said redox reagent comprises an oxidizing agent and a reducing agent.

36. The method of claim 25, wherein the refolding is performed at a temperature in the range of -10 °C to 60 °C.

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37. The method of claim 25, wherein the refolding is performed at a pH in the range of 5 to 12.

38. The method of claim 25, wherein the peptide is immobilized on a solid support.

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39. The method of claim 1, wherein a pair of cysteine residues is replaced pairwise with isoteric lactam or ester-thioether replacements

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40. The method of claim 39, wherein said replacement is selected from the group consisting of Ser/(Glu or Asp), Lys/(Glu or Asp), Cys/(Glu or Asp) and Cys/Ala combinations.

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41. The method of claim 13, wherein a pair of cysteine residues is replaced pairwise with isoteric lactam or ester-thioether replacements

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42. The method of claim 41, wherein said replacement is selected from the group consisting of Ser/(Glu or Asp), Lys/(Glu or Asp), Cys/(Glu or Asp) and Cys/Ala combinations.

43. The method of claim 25, wherein a pair of cysteine residues is replaced pairwise with isoteric lactam or ester-thioether replacements

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44. The method of claim 43, wherein said replacement is selected from the group consisting of Ser/(Glu or Asp), Lys/(Glu or Asp), Cys/(Glu or Asp) and Cys/Ala combinations.